



(19)

**Europäisches Patentamt
European Patent Office
Office européen des brevets**



(11) EP 0 495 257 B1

(12)

EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention
of the grant of the patent:
12.06.2002 Bulletin 2002/24

(51) Int Cl.7: **C11D 3/386**, C12N 9/42,
C12N 15/56, C11D 17/06

(21) Application number: 91202879.2

(22) Date of filing: 06.11.1991

(54) Compact detergent compositions with high activity cellulase

Kompakte Waschmittelzusammensetzungen mit hochaktiven Cellulasen

Compositions de détergent compactes contenant de la cellulase de haute activité

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IT LI LU NL SE

- Ceulemans, Raphael Angeline Alfons
B-3210 Lubbeek (Linde) (BE)

(30) Priority: 16.01.1991 EP 91870006

(74) Representative: Canonici, Jean-Jacques et al
Procter & Gamble European Technical Center
N.V.
Temselaan 100
1853 Strombeek-Bever (BE)

(43) Date of publication of application:
22.07.1992 Bulletin 1992/30

(56) References cited:
EP-A- 0 220 016
EP-A- 0 367 339
WO-A-89/09259
GB-A- 2 075 028

(73) Proprietor: THE PROCTER & GAMBLE COMPANY
Cincinnati, Ohio 45202 (US)

(72) Inventors:

- Baeck, André Cesar
B-2820 Bonheiden (BE)
- Busch, Alfred
B-2910 Londerzeel (BE)

EP 0 495 257 B1

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

DescriptionTechnical Field

5 [0001] The present invention concerns cellulase-containing granular detergent compositions which are in a "compact" form, i.e. they are of a relatively high density and contain a relatively low amount of inorganic filler salt, compared to conventional detergent compositions. In the detergent compositions herein the cellulase comprises a cellulase of high activity defined by the C14CMC method described herein. Preferably the cellulase is a specific single-component endoglucanase.

10 Background of the Invention

[0002] The need for detergent compositions which exhibit not only good cleaning properties, but also good fabric-softening performance, and other fabric care benefits, is well-established in the art.

15 [0003] The efficiency of cellulolytic enzymes, i.e. cellulases, in terms of textile cleaning and harshness-reducing agent for fabrics has been recognized for some time; GB-A-2,075,028, GB-A-2,095,275 and GB-A-2,094,826, disclose detergent compositions with cellulase for improved cleaning performance; GB-A-1,368,599 discloses the use of cellulase for reducing the harshness of cotton-containing fabrics; U.S. 4,435,307 teaches the use of a cellulolytic enzyme derived from *Humicola insolens* as well as a fraction thereof, designated ACXI, as a harshness-reducing detergent additive.

20 [0004] EP-A-0 269 168 discloses optimized detergent compositions containing cellulase, which are formulated at a mild alkaline pH range and provide combined fabric cleaning, fabric softening, and fabric care performance.

[0005] In WO 89109259 have been disclosed cellulase preparations useful for reducing the harshness of cotton-containing fabrics, comprising an endoglucanase component with a high endoase activity and affinity towards cellulose.

25 [0006] The practical exploitation of cellulases has however, been set back by the fact that cellulase preparations such as those disclosed in the above-mentioned prior art documents, are complex mixtures, of which only a certain fraction is effective in the fabric-care context; it was thus difficult to implement cost effective industrial production of cellulase for the detergent industry; and large quantities of such cellulase preparations would need to be applied, in order to obtain the desired effect on fabrics.

30 [0007] Improvements in cellulase production also often have not proven to be sufficiently identifiable in terms of applicability in detergents. Defining a cellulase selection criterium relevant for detergent application of cellulase was made possible by the C14CMC-method disclosed in EP-A-350 098. A minimum of 10% removal of immobilized radioactive labelled carboxymethylcellulose has been found to provide high activity cellulase. A preferred group of cellulase falling under the high activity definition according to the present invention has been disclosed in copending Danish Patent Application No. : 1159/90 filed May 5, 1990 (Priority document of WO 91/17243). There is disclosed a cellulase preparation consisting essentially of a homogeneous endoglucanase component which is immunoreactive with a monoclonal antibody raised against a partially purified 43kD cellulase derived from *Humicola insolens* DM1800.

35 [0008] The finding that this particular endoglucanase component of cellulase is advantageous for the treatment of cellulose-containing materials now permits to produce the cellulase cost-effectively, e.g. by employing recombinant DNA techniques, and allows to apply only a small quantity of the cellulase preparation, and obtain the desired effect on fabrics.

[0009] On the other hand, a new generation of detergent compositions is now being marketed, which can be best pictured as "compact detergents" although they have been given a variety of trade names such as "Ultra", "Supra", "Micro" ... The particularity of such detergent compositions is their relatively high density compared to conventional detergent compositions, and their ability to achieve the same efficiency than conventional detergent compositions by using a considerably lesser amount of "compact" detergent composition. This particularity is best reflected, in terms of composition, by a relatively low amount of inorganic filler salt. The efficiency of such "compact" detergent compositions is best achieved by eliminating the pre-wash cycle and by using dispersing and diffusing devices, which are put directly in the drum of the washing machine at the start of the main washing cycle.

45 [0010] It is an object of the present invention to provide detergent compositions in a compact form, having a relatively high density and containing a low amount of inorganic filler salt, which exhibit optimum cellulase efficiency.

[0011] In EP-A-381 397 has been disclosed the effect of low ionic-strength on enzyme performance, in particular lipase.

50 [0012] It has been surprisingly found however, that the effect of the compact matrix on the selected enzymes of the present invention is much higher than what could be expected from state of the art cellulases such as disclosed in EP-A-381 397.

[0013] It is another object of the present invention to provide a method for treating fabrics in a washing machine, comprising the utilization of the present detergent compositions at low levels, for the main wash cycle.

Summary of the Invention

[0014] The present invention relates to granular detergent compositions containing a surface-active agent, a builder, an enzyme, and if desired conventional additives, characterized in that the enzyme comprises a cellulase preparation providing at least 10% removal of immobilized radioactive labelled carboxymethylcellulose according to the C14CMC-method, at $25 \times 10^{-6}\%$ by weight of cellulase protein in the laundry test solution.

[0015] Preferably, the cellulase compound consists essentially of a homogeneous endoglucanase component which is immunoreactive with a monoclonal antibody raised against a partially purified about $\approx 43\text{kD}$ cellulase derived from Humicola insolens, DSM 1800, or which is homologous to said $\approx 43\text{kD}$ endoglucanase.

Detailed Description of the Invention

[0016] The present detergent compositions are in granular form and are characterized by their density, which is higher than the density of conventional detergent compositions. The density of the compositions herein ranges from 550 to 950g/liter, preferably 650 to 850 g/liter of composition, measured at 20°C.

[0017] The "compact" form of the compositions herein is best reflected, in terms of composition, by the amount of inorganic filler salt; inorganic filler salts are conventional ingredients of detergent compositions in powder form; In conventional detergent compositions, the filler salts are present in substantial amounts, typically 17-35% by weight of the total composition.

[0018] In the present compositions, the filler salt is present in amounts not exceeding 15% of the total composition, preferably not exceeding 10%, most preferably not exceeding 5% by weight of the composition.

[0019] Inorganic filler salts, such as meant in the present compositions are selected from the alkali and alkaline-earth-metal salts of sulphates and chlorides.

[0020] A preferred filler salt is sodium sulphate.

SURFACTANT

[0021] A wide range of surfactants can be used in the detergent compositions. A typical listing of anionic, nonionic, ampholytic and zwitterionic classes, and species of these surfactants, is given in US Patent 3,664,961 issued to Norris on May 23, 1972.

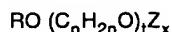
[0022] Mixtures of anionic surfactants are particularly suitable herein, especially mixtures of sulphonate and sulphate surfactants in a weight ratio of from 5:1 to 1:2, preferably from 3:1 to 2:3, more preferably from 3:1 to 1:1. Preferred sulphonates include alkyl benzene sulphonates having from 9 to 15, especially 11 to 13 carbon atoms in the alkyl radical, and alpha-sulphonated methyl fatty acid esters in which the fatty acid is derived from a C₁₂-C₁₈ fatty source preferably from a C₁₆-C₁₈ fatty source. In each instance the cation is an alkali metal, preferably sodium.

Preferred sulphate surfactants are alkyl sulphates having from 12 to 18 carbon atoms in the alkyl radical, optionally in admixture with ethoxy sulphates having from 10 to 20, preferably 10 to 16 carbon atoms in the alkyl radical and an average degree of ethoxylation of 1 to 6. Examples of preferred alkyl sulphates herein are tallow alkyl sulphate, coconut alkyl sulphate, and C₁₄₋₁₅ alkyl sulphates. The cation in each instance is again an alkali metal cation, preferably sodium.

[0023] One class of nonionic surfactants useful in the present invention are condensates of ethylene oxide with a hydrophobic moiety to provide a surfactant having an average hydrophilic-lipophilic balance (HLB) in the range from 8 to 17, preferably from 9.5 to 13.5, more preferably from 10 to 12.5. The hydrophobic (lipophilic) moiety may be aliphatic or aromatic in nature and the length of the polyoxyethylene group which is condensed with any particular hydrophobic group can be readily adjusted to yield a water-soluble compound having the desired degree of balance between hydrophilic and hydrophobic elements.

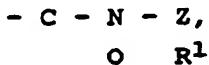
[0024] Especially preferred nonionic surfactants of this type are the C₉-C₁₅ primary alcohol ethoxylates containing 3-8 moles of ethylene oxide per mole of alcohol, particularly the C₁₄-C₁₅ primary alcohols containing 6-8 moles of ethylene oxide per mole of alcohol and the C₁₂-C₁₄ primary alcohols containing 3-5 moles of ethylene oxide per mole of alcohol.

[0025] Another class of nonionic surfactants comprises alkyl polyglucoside compounds of general formula



wherein Z is a moiety derived from glucose; R is a saturated hydrophobic alkyl group that contains from 12 to 18 carbon atoms; t is from 0 to 10 and n is 2 or 3; x is from 1.3 to 4, the compounds including less than 10% unreacted fatty alcohol and less than 50% short chain alkyl polyglucosides. Compounds of this type and their use in detergent are disclosed in EP-B 0 070 077, 0 075 996 and 0 094 118.

[0026] Also suitable as nonionic surfactants are poly hydroxy fatty acid amide surfactants of the formula R²



5

wherein R¹ is H, C₁₋₄ hydrocarbyl, 2-hydroxy ethyl, 2-hydroxy propyl or a mixture thereof, R₂ is C₅₋₃₁ hydrocarbyl, and Z is a polyhydroxyhydrocarbyl having a linear hydrocarbyl chain with at least 3 hydroxyls directly connected to the chain, or an alkoxylated derivative thereof. Preferably, R₁ is methyl, R₂ is a straight C₁₁₋₁₅ alkyl or alkenyl chain such as coconut alkyl or mixtures thereof, and Z is derived from a reducing sugar such as glucose, fructose, maltose, lactose, in a reductive amination reaction.

[0027] A further class of surfactants are the semi-polar surfactants such as amine oxides. Suitable amine oxides are selected from mono C₈-C₂₀, preferably C₁₀-C₁₄ N-alkyl or alkenyl amine oxides and propylene-1,3-diamine dioxides wherein the remaining N positions are substituted by methyl, hydroxyethyl or hydroxypropyl groups.

[0028] Another class of surfactants are amphoteric surfactants, such as polyamine-based species.

[0029] Cationic surfactants can also be used in the detergent compositions herein and suitable quaternary ammonium surfactants are selected from mono C₈-C₁₆, preferably C₁₀-C₁₄ N-alkyl or alkenyl ammonium surfactants wherein remaining N positions are substituted by methyl, hydroxyethyl or hydroxypropyl groups.

[0030] Mixtures of surfactant types are preferred, more especially anionic-nonionic and also anionic-nonionic-cationic mixtures. Particularly preferred mixtures are described in British Patent No. 2040987 and European Published Application No. 0 087 914. The detergent compositions can comprise from 1%-70% by weight of surfactant, but usually the surfactant is present in the compositions herein an amount of from 1% to 30%, more preferably from 10-25% by weight.

BUILDER

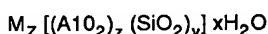
25

[0031] Builder materials will typically be present at from 10% to 60% of the detergent compositions herein. The compositions herein are free or substantially free of phosphate-containing builders (substantially free being herein defined to constitute less than 1% of the total detergent builder system), and the builder system herein consists of water-soluble builders, water-insoluble builders, or mixtures thereof.

[0032] Water insoluble builders can be an inorganic ion exchange material, commonly an inorganic hydrated aluminosilicate material, more particularly a hydrated synthetic zeolite such as hydrated Zeolite A, X, B or HS.

[0033] Preferred aluminosilicate ion-exchange materials have the unit cell formula

35

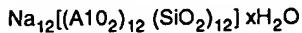


wherein M is a calcium-exchange cation, z and y are at least 6; the molar ratio of z to y is from 1.0 to 0.5 and x is at least 5, preferably from 7.5 to 276, more preferably from 10 to 264. The aluminosilicate materials are in hydrated form and are preferably crystalline containing from 10% to 28%, more preferably from 18% to 22% water.

[0034] The above aluminosilicate ion exchange materials are further characterized by a particle size diameter of from 0.1 to 10 micrometers, preferably from 0.2 to 4 micrometers. The term "particle size diameter" herein represents the average particle size diameter of a given ion exchange material as determined by conventional analytical techniques such as, for example, microscopic determination utilizing a scanning electron microscope. The aluminosilicate ion exchange materials are further characterized by their calcium ion exchange capacity, which is at least 200 mg equivalent of CaCO₃ water hardness/g of aluminosilicate, calculated on an anhydrous basis, and which generally is in the range of from 300 mg eq./g to 352 mg eq./g. The aluminosilicate ion exchange materials herein are still further characterized by their calcium ion exchange rate which is described in detail in GB-1,429,143.

[0035] Aluminosilicate ion exchange materials useful in the practice of this invention are commercially available and can be naturally occurring materials, but are preferably synthetically derived. A method for producing aluminosilicate ion exchange materials is discussed in US Patent No. 3,985,669. Preferred synthetic crystalline aluminosilicate ion exchange materials useful herein are available under the designation Zeolite A, Zeolite B, Zeolite X, Zeolite HS and mixtures thereof. In an especially preferred embodiment, the crystalline aluminosilicate ion exchange material is Zeolite A and has the formula

55



wherein x is from 20 to 30, especially 27. Zeolite X of formula Na₈₆ [(AlO₂)₈₆(SiO₂)₁₀₆] - 10 .276H₂O is also suitable,

as well as Zeolite HS of formula $\text{Na}_6[(\text{AlO}_2)_6(\text{SiO}_2)_6] \cdot 7.5 \text{ H}_2\text{O}$.

[0036] Another suitable water-insoluble, inorganic builder material is layered silicate, e.g. SKS-6 (Hoechst). SKS-6 is a crystalline layered silicate consisting of sodium silicate ($\text{Na}_2\text{Si}_2\text{O}_5$). The high $\text{Ca}^{++}/\text{Mg}^{++}$ binding capacity is mainly a cation exchange mechanism. In hot water, the material becomes more soluble.

5 [0037] The water-soluble builder can be a monomeric or oligomeric carboxylate chelating agent.

[0038] Suitable carboxylates containing one carboxy group include lactic acid, glycollic acid and ether derivatives thereof as disclosed in Belgian Patent Nos. 831,368, 821,369 and 821,370. Polycarboxylates containing two carboxy groups include the water-soluble salts of succinic acid, malonic acid, (ethylenedioxy) diacetic acid, maleic acid, diglycollic acid, tartaric acid, tartronic acid and fumaric acid, as well as the ether carboxylates described in German Offenlegungsschrift 2,446,686, and 2,446,687 and U.S. Patent No. 3,935,257 and the sulfinyl carboxylates described in Belgian Patent No. 840,623. Polycarboxylates containing three carboxy groups include, in particular, water-soluble citrates, aconitlates and citraconates as well as succinate derivatives such as the carboxymethyloxysuccinates described in British Patent No. 1,379,241, lactoxysuccinates described in Netherlands Application 7205873, and the oxypolycarboxylate materials such as 2-oxa-1,1,3-propane tricarboxylates described in British Patent No. 1,387,447.

10 [0039] Polycarboxylates containing four carboxy groups include oxydisuccinates disclosed in British Patent No. 1,261,829, 1,1,2,2-ethane tetracarboxylates, 1,1,3,3-propane tetracarboxylates and 1,1,2,3-propane tetracarboxylates. Polycarboxylates containing sulfo substituents include the sulfosuccinate derivatives disclosed in British Patent Nos. 1,398,421 and 1,398,422 and in U.S. Patent No. 3,936,448, and the sulfonated pyrolysed citrates described in British Patent No. 1,082,179, while polycarboxylates containing phosphone substituents are disclosed in British Patent

15 No. 1,439,000.

[0040] Alicyclic and heterocyclic polycarboxylates include cyclopentane-cis,cis,cis-tetracarboxylates, cyclopentadiene pentacarboxylates, 2,3,4,5-tetrahydrofuran - cis, cis, cis-tetracarboxylates, 2,5-tetrahydrofuran -cis - dicarboxylates, 2,2,5,5-tetrahydrofuran - tetracarboxylates, 1,2,3,4,5,6-hexane -hexacarboxylates and carboxymethyl derivatives of polyhydric alcohols such as sorbitol, mannitol and xylitol. Aromatic polycarboxylates include mellitic acid, pyromellitic acid and the phthalic acid derivatives disclosed in British Patent No. 1,425,343.

[0041] Of the above, the preferred polycarboxylates are hydroxycarboxylates containing up to three carboxy groups per molecule, more particularly citrates.

[0042] Preferred builder systems for use in the present compositions include a mixture of a water-insoluble aluminosilicate builder such as zeolite A, and a water-soluble carboxylate chelating agent such as citric acid.

20 [0043] Other builder materials that can form part of the builder system for the purposes of the invention include inorganic materials such as alkali metal carbonates, bicarbonates, silicates, and organic materials such as the organic phosphonates, amino polyalkylene phosphonates and amino polycarboxylates.

[0044] Other suitable water-soluble organic salts are the homo- or co-polymeric acids or their salts, in which the polycarboxylic acid comprises at least two carboxyl radicals separated from each other by not more than two carbon atoms.

25 [0045] Polymers of this type are disclosed in GB-A-1,596,756. Examples of such salts are polyacrylates of MW 2000-5000 and their copolymers with maleic anhydride, such copolymers having a molecular weight of from 20,000 to 70,000, especially about 40,000.

40 CELLULASE

[0046] The activity of enzymes and particularly the activity of cellulase enzyme has been defined for various applications by different analytical methods. These methods all attempt to provide a realistic assessment of the expected in use performance or at least a measurement correlating with the in use performance. As has been detailed in European Patent Application EP-A-350098, many of the methods, particularly those frequently used by cellulase manufacturers, are not sufficiently correlated with the in use performance of cellulase in laundry detergent compositions. This is due to the various other usage conditions for which these activity measurement methods have been developed.

45 [0047] The method described in EP-A-350098, has been developed to be and to have a predictive correlation for the ranking of cellulase activity in laundry detergent compositions.

50 [0048] The present invention therefore uses the method disclosed in EP-A-350098 to screen cellulases in order to distinguish cellulases which are useful in the present invention and those which would not provide the objectives of the present invention. The screening method, hereinafter referred to as CI4CMC-Method, which has been adopted from the method disclosed in EP-A-350098, can be described as follows :

55 Principle :

[0049] The principle of the CI4CMC-Method for screening is to measure at a defined cellulase concentration in a wash solution the removal of immobilized carboxy methyl cellulose (CMC) from a cloth substrate. The removal of CMC

is measured by radio-active labelling of some of the CMC by using C14 radio-active carbon. Simple counting of the amount of radio-active C14 on the cloth substrate before and after the cellulase treatment allows the evaluation of the cellulase activity.

5 Sample preparation :

[0050] **CMC preparation :** The radio-active CMC stock solution is prepared according to Table I. The radio-active CMC can be obtained by methods referred to in EP-A-350098.

10 [0051] **Fabric substrates :** The fabric substrates are muslin cotton swatches having a size of 5 cm x 5 cm. They are inoculated with 0.35 ml of the radio-active labelled CMC stock solution in their center. The muslin cotton swatches are then air dried.

15 [0052] **Immobilization of CMC :** To immobilize the radio-active labelled CMC on the muslin cotton swatches, laundero-meter equipment " Linitest Original Haunau " made by Original Haunau, Germany, is used. A metal jar of the laundero-meter is filled with 400 ml of hard water (4 mmol/liter of Ca⁺⁺ ions). A maximum number of 13 swatches can be used per jar. The jar is then incubated in a heat-up cycle from 20°C to 60°C over 40 minutes in the laundero-meter equipment. After incubation the swatches are rinsed under running city water for 1 minute. They are squeezed and allowed to air dry for at least 30 minutes.

20 [0053] According to EP-A-350098 samples of the swatches with immobilized radio-active CMC can also be measured as "blank samples" without washing.

25 Sample treatment :

[0054] **Laundry test solution :** The laundry test solution is prepared according to the composition of Table II. It is balanced to pH 7.5. The laundry test solution is the basis to which a cellulase test sample is added. Care should be taken to not dilute the laundry test solution by adding water to a 100% balance prior to having determined the amount of cellulase to be added. The amount of cellulase which is used in this screening test should be added to provide 25 x 10⁻⁶ weight percent of cellulase protein in the laundry test solution (equivalent to 0.25 milligram/liter at 14.5 °C).

30 [0055] **Wash procedure :** The swatches thus inoculated with radio-active labelled CMC are then treated in a laundry simulation process. The laundry process is simulated in the laundero-meter type equipment, " Linitest, Original Haunau ", by Original Haunau, Haunau Germany. An individual swatch is put into a 20 cm³ glass vial. The vial is filled with 10 ml of the laundry test solution and then sealed liquid tight. Up to 5 vials are put into each laundero-meter jar. The jar is filled with water as a heat transfer medium for the laundering simulation. The laundering simulation is conducted as a heat-up cycle from 20°C to 60°C over 40 minutes.

35 [0056] After the processing of the samples the vials are submerged in cold water and subsequently each swatch is taken out of its vial, rinsed in a beaker under running soft water, squeezed and allowed to air dry for at least 30 minutes.

Measurement :

40 [0057] In order to measure radio-active labelled CMC removal, a scintillation counter, for example, a LKB 1210 Ultrabeta Scintillation Counter, is used. In order to obtain most accurate results, the instruction manual for optimum operation of the particular scintillation counter should be followed. For example, for the LKB 1210 Ultrabeta Scintillation Counter, the following procedure should be followed. The swatch to be measured is put into a plastic vial filled with 12 ml of scintillator liquid (e.g. scintillator 299 from Packard). The swatch is then allowed to stabilize for at least 30 minutes. The vial is then put into the LKB 1210 Ultrabeta Scintillation Counter and the respective radio-activity counts for the swatch is obtained.

45 [0058] In order to measure the amount of CMC removal due only to the cellulase, a measurement of a swatch which has been inoculated at the same time but has been treated in the laundry test solution without cellulase, is necessary. The activity of the cellulase is then expressed as percent of radio-active labelled CMC removal. This percentage is calculated by the following formula :

50

$$\% \text{ of radio-active CMC removal} = \frac{X_O - X_C}{X_O} \times 100$$

Wherein

55

XO is the radioactivity scintillation count of a swatch treated with the laundry test solution without cellulase

XC is the radioactivity scintillation count of a swatch treated with the laundry test solution containing the cellulase to be evaluated

Statistical considerations, procedure confirmation :

[0059] In order to provide statistically sound results, standard statistical analysis should be employed. For the given example, using the LKB 1210 Ultrabeta Scintillation Counter, it has been found that a sample size of 3 swatches for each radioactivity scintillation count can be used.

[0060] In order to confirm the procedure by internal crosschecking, measurement and calculation of the "blank sample" according to EP-A-350098 are recommended. This will allow to detect and eliminate errors.

Interpretation of results :

[0061] The described screening test does provide a fast, unique and reliable method to identify cellulases which satisfy the activity criteria of the present invention versus cellulases which are not part of the present invention.

[0062] It has been found that a removal of 10% or more of the immobilized radioactive labelled CMC according to the above C14CMC-method, indicates that the respective cellulase satisfies the requirements of the invention.

[0063] It will be obvious to those skilled in the art that removal percentages above 10% indicate a higher activity for the respective cellulase. It therefore is contemplated that cellulase providing above 25% or preferably above 50% removal of radioactive labelled CMC, at the protein concentration in the laundry test solution according to the C14CMC-method, would provide indication of an even better performance of the cellulase for use in laundry detergents.

[0064] It also has been contemplated that usage of higher concentrations of cellulase for C14CMC-method, would provide higher removal percentages. However, there exists no linear proven correlation between cellulase concentration and removal percentage obtained by it.

[0065] It also has been contemplated that usage of higher concentrations of cellulase for C14CMC-method, would provide higher removal percentages.

TABLE I :

Radioactive C ₁₄ labelled CMC stock solution (all percentages by weight of total solution)	
Total CMC*	99.2 x 10 ⁻³ %
Ethanol	14985.12 x 10 ⁻³ %
Deionized Water	84915.68 x 10 ⁻³ %
Total :	100%

* Total CMC contains non-radio-active and radio-active CMC to provide a radio-activity which allows sufficiently clear readings on the scintillation counter used. For example, the radio-active CMC can have an activity of 0.7 millicurie/g and be mixed

TABLE II :

Laundry test solution (all percentages by weight of total solution)	
Linear C ₁₂ alkyl benzene sulphonlic acid	0.110%
Coconut alkyl sulphate (TEA salt)	0.040%
C ₁₂₋₁₅ alcohol ethoxylate (E07)	0.100%
Coconut fatty acid	0.100%
Oleic acid	0.050%
Citric acid	0.010%
Triethanolamine	0.040%
Ethanol	0.060%
Propanediol	0.015%
Sodium hydroxide	0.030%
Sodium formate	0.010%

TABLE II : (continued)

Laundry test solution (all percentages by weight of total solution)	
5 Protease	0.006%
Water (2.5 mmol/liter Ca ⁺⁺), pH adjustment agent (HCl or NaOH solutions) and cellulase	balance to 100%

[0066] According to the present invention, preferred cellulases are those as described in Danish Patent Application 1159/90 (Priority document of WO 91/17243). For example, a cellulase preparation useful in the compositions of the invention can consist essentially of a homogeneous endoglucanase component, which is immunoreactive with an antibody raised against a highly purified 43kD cellulase derived from Humicola insolens, DSM 1800, or which is homologous to said 43kD endoglucanase.

[0067] It should be stressed that all cellulase enzymes according to the present invention have to meet the criteria of the above mentioned screening test. However, in the Danish Patent Application 1159/90 (Priority document of WO 91/17243) additional criteria are established allowing to identify preferred cellulase enzymes in combination with the present screening test.

[0068] Cellulase preparations particularly useful in the compositions of the invention are those in which in addition to the screening test, the endoglucanase component exhibits a CMC-endoase activity of at least about 50, preferably at least about 60, in particular at least about 90 CMC-endoase units per mg of total protein. In particular, a preferred endoglucanase component exhibits a CMC-endoase activity of at least 100 CMC-endoase units per mg of total protein.

[0069] In the present context, the term "CMC-endoase activity" refers to the endoglucanase activity of the endoglucanase component in terms of its ability to degrade cellulose to glucose, cellobiose and triose, as determined by a viscosity decrease of a solution of carboxymethyl cellulose (CMC) after incubation with the cellulase preparation of the invention, as described in detail below.

[0070] The CMC-endoase (endoglucanase) activity can be determined from the viscosity decrease of CMC, as follows : A substrate solution is prepared, containing 35 g/l CMC (Hercules 7 LFD) in 0.1 M tris buffer at pH 9.0. The enzyme sample to be analyzed is dissolved in the same buffer, 10 ml substrate solution and 0.5 ml enzyme solution are mixed and transferred to a viscosimeter (e.g. Haake VT 181, NV sensor, 181 rpm), thermostated at 40°C. Viscosity readings are taken as soon as possible after mixing and again 30 minutes later. The amount of enzyme that reduces the viscosity to one half under these conditions is defined as 1 unit of CMC-endoase activity.

[0071] SDS polyacrylamide gel electrophoresis (SDS-PAGE) and isoelectric focusing with marker proteins in a manner known to persons skilled in the art were used to determine the molecular weight and isoelectric point (pi), respectively, of the endoglucanase component in the cellulase preparation useful in the present context. In this way, the molecular weight of a specific endoglucanase component was determined to be 43kD. The isoelectric point of this endoglucanase was determined to be about 5.1.

[0072] The cellobiohydrolase activity may be defined as the activity towards cellobiose p-nitrophenyl. The activity is determined as umole nitrophenyl released per minute at 37°C and pH 7.0. The present endoglucanase component was found to have essentially no cellobiohydrolase activity.

[0073] The endoglucanase component in the cellulase preparation herein has initially been isolated by extensive purification procedures, i.a. involving reverse phase HPLC purification of a crude H. insolens cellulase mixture according to U.S. 4,435,307. This procedure has surprisingly resulted in the isolation of a 43kD endoglucanase as a single component with unexpectedly favourable properties due to a surprisingly high endoglucanase activity.

[0074] Also, in addition to the screening test, the cellulase enzymes useful in the present compositions can further be defined as enzymes exhibiting endoglucanase activity (in the following referred to as an "endoglucanase enzyme"), which enzymes have the amino acid sequence shown in the appended Sequence Listing ID#2, or a homologue thereof exhibiting endoglucanase activity.

[0075] In the present context, the term "homologue" is intended to indicate a polypeptide encoded by DNA which hybridizes to the same probe as the DNA coding for the endoglucanase enzyme with this amino acid sequence under certain specified conditions (such as presoaking in 5xSSC and prehybridizing for 1 h at 40°C in a solution of 20% formamide, 5xDenhardt's solution, 50 mM sodium phosphate, pH 6.8, and 50 ug of denatured sonicated calf thymus DNA, followed by hybridization in the same solution supplemented with 100 uM ATP for 18 h at 40°C). The term is intended to include derivatives of the aforementioned sequence obtained by addition of one or more amino acid residues to either or both the C- and N-terminal of the native sequence, substitution of one or more amino acid residues at one or more sites in the native sequence, deletion of one or more amino acid residues at either or both ends of the native amino acid sequence or at one or more sites within the native sequence, or insertion of one or more amino acid residues at one or more sites in the native sequence.

[0076] The endoglucanase enzyme herein may be one producible by species of Humicola such as Humicola insolens

e.g. strain DSM 1800, deposited on October 1, 1981 at the Deutsche Sammlung von Mikroorganismen, Mascheroder Weg 1B, D-3300 Braunschweig, FRG, in accordance with the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure (the Budapest Treaty).

5 [0077] In still a further aspect, the cellulase enzymes useful herein can be defined, in addition to the screening test, as endoglucanase enzymes which have the amino acid sequence shown in the appended Sequence Listing ID#4, or a homologue thereof (as defined above) exhibiting endoglucanase activity. Said endoglucanase enzyme may be one producible by a species of Fusarium, such as Fusarium oxysporum, e.g. strain DSM 2672, deposited on June 6, 1983 at the Deutsche Sammlung von Mikroorganismen, Mascheroder Weg 1B, D-3300 Braunschweig, FRG, in accordance with the provisions of the Budapest Treaty.

10 [0078] Furthermore, it is contemplated that homologous endoglucanases may be derived from other microorganisms producing cellulolytic enzymes, e.g. species of Trichoderma, Mycelopithora, Phanerochaete, Schizophyllum, Penicillium, Aspergillus, and Geotrichum.

15 [0079] For industrial production of the cellulase preparation herein, however, it is preferred to employ recombinant DNA techniques or other techniques involving adjustments of fermentations or mutation of the microorganisms involved to ensure overproduction of the desired enzymatic activities. Such methods and techniques are known in the art and may readily be carried out by persons skilled in the art.

20 [0080] The endoglucanase component may thus be one which is producible by a method comprising cultivating a host cell transformed with a recombinant DNA vector which carries a DNA sequence encoding said endoglucanase component or a precursor of said endoglucanase component as well as DNA sequences encoding functions permitting the expression of the DNA sequence encoding the endoglucanase component or precursor thereof, in a culture medium under conditions permitting the expression of the endoglucanase component or precursor thereof and recovering the endoglucanase component from the culture.

25 [0081] DNA constructs comprising a DNA sequence encoding an endoglucanase enzyme as described above, or a precursor form of the enzyme, include the DNA constructs having a DNA sequence as shown in the appended Sequence Listings ID#1 or ID#3, or a modification thereof. Examples of suitable modifications of the DNA sequence are nucleotide substitutions which do not give rise to another amino acid sequence of the endoglucanase, but which correspond to the codon usage of the host organism into which the DNA construct is introduced or nucleotide substitutions which do give rise to a different amino acid sequence and therefore, possibly, a different protein structure which might give rise to an endoglucanase mutant with different properties than the native enzyme. Other examples of possible 30 modifications are insertion of one or more nucleotides at either end of the sequence, or deletion of one or more nucleotides at either end or within the sequence.

35 [0082] DNA constructs encoding endoglucanase enzymes useful herein may be prepared synthetically by established standard methods, e.g. the phosphoamidite method described by S.L. Beaucage and M.H. Caruthers, Tetrahedron Letters 22, 1981, pp. 1859-1869, or the method described by Matthes et al., EMBO Journal 3, 1984, pp. 801-805. According to the phosphoamidite method, oligonucleotides are synthesized, e.g. in an automatic DNA synthesizer, purified, annealed, ligated and cloned in suitable vectors.

40 [0083] A DNA construct encoding the endoglucanase enzyme or a precursor thereof may, for instance, be isolated by establishing a cDNA or genomic library of a cellulase-producing microorganism, such as Humicola insolens, DSM 1800, and screening for positive clones by conventional procedures such as by hybridization using oligonucleotide probes synthesized on the basis of the full or partial amino acid sequence of the endoglucanase in accordance with standard techniques (cf. Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd. Ed. Cold Spring Harbor, 1989), or by selecting for clones expressing the appropriate enzyme activity (i.e. CMC-endopeptidase activity as defined above), or by selecting for clones producing a protein which is reactive with an antibody against a native cellulase (endoglucanase).

45 [0084] Finally, the DNA construct may be of mixed synthetic and genomic, mixed synthetic and cDNA or mixed genomic and cDNA origin prepared by ligating fragments of synthetic, genomic or cDNA origin (as appropriate), the fragments corresponding to various parts of the entire DNA construct, in accordance with standard techniques. The DNA construct may also be prepared by polymerase chain reaction using specific primers, for instance as described in US 4,683,202 or R.K. Saiki et al., Science 239, 1988, pp. 487-491.

50 [0085] Recombinant expression vectors into which the above DNA constructs are inserted include any vector which may conveniently be subjected to recombinant DNA procedures, and the choice of vector will often depend on the host cell into which it is to be introduced. Thus, the vector may be an autonomously replicating vector, i.e. a vector which exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, e.g. a plasmid. Alternatively, the vector may be one which, when introduced into a host cell, is integrated into the host cell genome and replicated together with the chromosome(s) into which it has been integrated.

55 [0086] In the vector, the DNA sequence encoding the endoglucanase should be operably connected to a suitable promoter and terminator sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the

host cell. The procedures used to ligate the DNA sequences coding for the endoglucanase, the promoter and the terminator, respectively, and to insert them into suitable vectors are well known to persons skilled in the art (cf., for instance, Sambrook et al., *op.cit.*).

[0087] Host cells which are transformed with the above DNA constructs or the above expression vectors may be for instance belong to a species of *Aspergillus*, most preferably *Aspergillus oryzae* or *Aspergillus niger*. Fungal cells may be transformed by a process involving protoplast formation and transformation of the protoplasts followed by regeneration of the cell wall in a manner known per se. The use of *Aspergillus* as a host microorganism is described in EP 238 023 (of Novo Industri A/S). The host cell may also be a yeast cell, e.g. a strain of *Saccharomyces cerevisiae*.

[0088] Alternatively, the host organism may be a bacterium, in particular strains of *Streptomyces* and *Bacillus*, and *E. coli*. The transformation of bacterial cells may be performed according to conventional methods, e.g. as described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, 1989.

[0089] The screening of appropriate DNA sequences and construction of vectors may also be carried out by standard procedures, cf. Sambrook et al., *op.cit.*

[0090] The medium used to cultivate the transformed host cells may be any conventional medium suitable for growing the host cells in question. The expressed endoglucanase may conveniently be secreted into the culture medium and may be recovered therefrom by well-known procedures including separating the cells from the medium by centrifugation or filtration, precipitating proteinaceous components of the medium by means of a salt such as ammonium sulphate, followed by chromatographic procedures such as ion exchange chromatography, affinity chromatography, or the like.

[0091] By employing recombinant DNA techniques as indicated above, techniques of protein purification, techniques of fermentation and mutation or other techniques which are well known in the art, it is possible to provide endoglucanases of a high purity.

[0092] The level in the present composition of cellulase described above should be such that the amount of enzyme protein to be delivered in the wash solution is from 0.005 to 40 mg/liter of wash solution, preferably 0.01 to 10 mg/liter of wash solution.

25

OPTIONAL INGREDIENTS

[0093] The present compositions will typically include optional ingredients that normally form part of detergent compositions Antiredeposition and soil suspension agents, optical brighteners, bleaches, bleach activators, suds suppressors, anticaking agents, dyes and pigments are examples of such optional ingredients and can be added in varying amounts as desired.

[0094] Antiredeposition and soil suspension agents suitable herein include cellulose derivatives such as methylcellulose, carboxymethylcellulose and hydroxyethylcellulose, and homo- or co-polymeric polycarboxylic acids or their salts. Polymers of this type include the polyacrylates and maleic anhydride-acrylic acid copolymers previously mentioned as builders, as well as copolymers of maleic anhydride with ethylene, methylvinyl ether or methacrylic acid, the maleic anhydride constituting at least 20 mole percent of the copolymer. These materials are normally used at levels of from 0.5% to 10% by weight, more preferably from 0.75% to 8%, most preferably from 1% to 6% by weight of the composition.

[0095] Preferred optical brighteners are anionic in character, examples of which are disodium 4,4'-bis-(2-diethanolamino-4-anilino -s- triazin-6-ylamino)stilbene-2:2¹ disulphonate, disodium 4, - 4'-bis-(2-morpholino-4-anilino-s-triazin-6-ylaminostilbene-2:2¹ - disulphonate, disodium 4,4' - bis-(2,4-dianilino-s-triazin-6-ylamino)stilbene-2:2¹ - disulphonate, monosodium 4,4'-bis-(2,4-dianilino-s-triazin-6-ylamino)stilbene-2-sulphonate, disodium 4,4'-bis-(2-anilino-4-(N-methyl-N-2-hydroxyethylamino)-s-triazin-6-ylamino)stilbene-2,2¹ - disulphonate, disodium 4,4'-bis-(4-phenyl-2,1,3-triazol-2-yl)-stilbene-2,2¹ disulphonate, disodium 4,4'-bis(2-anilino-4-(1-methyl-2-hydroxyethylamino)-s-triazin-6-ylamino)stilbene-2,2¹disulphonate and sodium 2(stilbyl-4¹¹-(naphtho-1,2:4,5)-1,2,3 - triazole-2¹¹-sulphonate.

[0096] Any particulate inorganic perhydrate bleach can be used, in an amount of from 3% to 40% by weight, more preferably from 8% to 25% by weight and most preferably from 12% to 20% by weight of the compositions. Preferred examples of such bleaches are sodium perborate monohydrate and tetrahydrate, percarbonate, and mixtures thereof.

[0097] Another preferred separately mixed ingredient is a peroxy carboxylic acid bleach precursor, commonly referred to as a bleach activator, which is preferably added in a prilled or agglomerated form. Examples of suitable compounds of this type are disclosed in British Patent Nos. 1586769 and 2143231 and a method for their formation into a prilled form is described in European Published Patent Application No. 0 062 523. Preferred examples of such compounds are tetracetyl ethylene diamine and sodium 3, 5, 5 trimethyl hexanoyloxybenzene sulphonate.

[0098] Bleach activators are normally employed at levels of from 0.5% to 10% by weight, more frequently from 1% to 8% and preferably from 2% to 6% by weight of the composition.

[0099] Another optional ingredient is a suds suppressor, exemplified by silicones, and silica-silicone mixtures. Silicones can be generally represented by alkylated polysiloxane materials while silica is normally used in finely divided forms exemplified by silica aerogels and xerogels and hydrophobic silicas of various types. These materials can be

incorporated as particulates in which the suds suppressor is advantageously releasably incorporated in a water-soluble or water-dispersible, substantially non-surface-active detergent impermeable carrier. Alternatively the suds suppressor can be dissolved or dispersed in a liquid carrier and applied by spraying on to one or more of the other components.

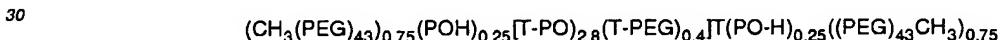
5 [0100] As mentioned above, useful silicone suds controlling agents can comprise a mixture of an alkylated siloxane, of the type referred to hereinbefore, and solid silica. Such mixtures are prepared by affixing the silicone to the surface of the solid silica. A preferred silicone suds controlling agent is represented by a hydrophobic silanated (most preferably trimethyl-silanated) silica having a particle size in the range from 10 millimicrons to 20 millimicrons and a specific surface area above 50 m²/g intimately admixed with dimethyl silicone fluid having a molecular weight in the range from about 500 to about 200,000 at a weight ratio of silicone to silanated silica of from about 1:1 to about 1:2.

10 [0101] A preferred silicone suds controlling agent is disclosed in Bartolotta et al. U.S. Patent 3,933,672. Other particularly useful suds suppressors are the self-emulsifying silicone suds suppressors, described in German Patent Application DTOS 2,646,126 published April 28, 1977. An example of such a compound is DC-544, commercially available from Dow Corning, which is a siloxane/glycol copolymer.

15 [0102] The suds suppressors described above are normally employed at levels of from 0.001% to 2% by weight of the composition, preferably from 0.01% to 1% by weight. The incorporation of the suds modifiers is preferably made as separate particulates, and this permits the inclusion therein of other suds controlling materials such as C20-C24 fatty acids, microcrystalline waxes and high MW copolymers of ethylene oxide and propylene oxide which would otherwise adversely affect the dispersibility of the matrix. Techniques for forming such suds modifying particulates are disclosed in the previously mentioned Bartolotta et al U.S. Patent No. 3,933,672.

20 [0103] Other useful polymeric materials are the polyethylene glycols, particularly those of molecular weight 1000-10000, more particularly 2000 to 8000 and most preferably about 4000. These are used at levels of from 0.20% to 5% more preferably from 0.25% to 2.5% by weight. These polymers and the previously mentioned homo- or co-polymeric polycarboxylate salts are valuable for improving whiteness maintenance, fabric ash deposition, and cleaning performance on clay, proteinaceous and oxidizable soils in the presence of transition metal impurities.

25 [0104] Soil release agents useful in compositions of the present invention are conventionally copolymers or terpolymers of terephthalic acid with ethylene glycol and/or propylene glycol units in various arrangements. Examples of such polymers are disclosed in the commonly assigned US Patent Nos. 4116885 and 4711730 and European Published Patent Application No. 0 272 033. A particular preferred polymer in accordance with EP-A-0 272 033 has the formula



where PEG is -(OC₂H₄)O-, PO is (OC₃H₆O) and T is (pcOC₆H₄CO).

35 [0105] Certain polymeric materials such as polyvinyl pyrrolidones typically of MW 5000-20000, preferably 10000-15000, also form useful agents in preventing the transfer of labile dyestuffs between fabrics during the washing process.

40 [0106] Fabric softening agents can also be incorporated into detergent compositions in accordance with the present invention. These agents may be inorganic or organic in type. Inorganic softening agents are exemplified by the smectite clays disclosed in GB-A-1,400,898. Organic fabric softening agents include the water-insoluble tertiary amines as disclosed in GB-A-1514276 and EP-B-0 011 340 and their combination with mono C12-C14 quaternary ammonium salts are disclosed in EP-B-0 026 527 and EP-B-0 026 528 and di-long-chain amides as disclosed in EP-B-0 242 919. Other useful organic ingredients of fabric softening systems include high molecular weight polyethylene oxide materials as disclosed in EP-A-0 299 575 and 0 313 146.

45 [0107] Levels of smectite clay are normally in the range from 5% to 20%, more preferably from 8% to 15% by weight with the material being added as a dry mixed component to the remainder of the formulation. Organic fabric softening agents such as the water-insoluble tertiary amines or di-long-chain amide materials are incorporated at levels of from 0.5% to 5% by weight, normally from 1% to 3% by weight whilst the high molecular weight polyethylene oxide materials and the water-soluble cationic materials are added at levels of from 0.1% to 2%, normally from 0.15% to 1.5% by weight. These materials are normally added to the spray dried portion of the composition, although in some instances it may be more convenient to add them as a dry mixed particulate, or spray them as a molten liquid on to other solid components of the composition.

50 [0108] Enzymes other than the specific cellulase preparation herein can be present in the composition herein, such as proteases, lipases and amylases.

55 MAKING PROCESS

[0109] Compositions according to the present invention can be made via a variety of methods including dry mixing, spray drying, agglomeration and granulation and combinations of any of these techniques.

PREFERRED MAKING PROCESS

[0110] A preferred method of making the compositions herein involves a combination of spray drying, agglomeration in a high speed mixer and dry mixing.

5 [0111] A first granular component containing a relatively insoluble anionic surfactant is spray dried and part of the spray dried product is diverted and subjected to a low level of nonionic surfactant spray on before being reblended with the remainder. A second granular component is made by dry neutralisation of an anionic surfactant acid using sodium carbonate as the neutralising agent in a continuous high speed blender such as a Lodige KM mixer. The first and second components together with other dry mix ingredients such as the carboxylate chelating agent, inorganic 10 peroxygen bleach, bleach activator, soil suspension agent, silicate and enzyme are then fed to a conveyor belt from which they are transferred to a horizontally rotating drum in which perfume and silicone suds suppressor are sprayed on to the product. In highly preferred compositions, a further drum mixing step is employed in which a low (approx. 2%) level of finely divided crystalline aluminosilicate is introduced to increase density and improve granular flow characteristics.

15 PROCESS OF WASHING

20 [0112] The compact detergent compositions herein have the ability to achieve the same efficiency than conventional detergent compositions, when a considerably lesser amount of composition herein, is used in the main wash cycle of a washing machine.

[0113] Accordingly, in an other embodiment of the invention, it is herewith provided for a process for washing fabrics in a washing machine wherein an amount of from 15 to 170 g of a detergent composition according to the present invention is used for the main wash cycle.

25 [0114] Typically, under European conditions, the recommended usage is from 80 to 140 g of detergent composition for the main wash cycle, without the need of a pre-wash.

[0115] The detergent compositions herein are preferably delivered directly to the drum and not indirectly via the outer casing of the machine. This can most easily be achieved by incorporation of the composition in a bag or container from which it can be released at the start of the wash cycle in response to agitation, a rise in temperature or immersion in the wash water in the drum. Such a container will be placed in the drum, together with the fabrics to be washed.

30 [0116] Alternatively the washing machine itself may be adapted to permit direct addition of the composition to the drum e.g. by a dispensing arrangement in the access door.

[0117] Products comprising a detergent composition enclosed in a bag or container are usually designed in such a way that container integrity is maintained in the dry state to prevent egress of the contents when dry, but are adapted for release of the container contents on exposure to a washing environment, normally on immersion in an aqueous solution.

35 [0118] Usually the container will be flexible, such as a bag or pouch. The bag may be of fibrous construction coated with a water impermeable protective material so as to retain the contents, such as is disclosed in European published Patent Application No. 0 018 678. Alternatively it may be formed of a water insoluble synthetic polymeric material provided with an edge seal or closure designed to rupture in aqueous media as disclosed in European published Patent Application Nos. 0 011 500, 0 011 501, 0 011 502, and 0 011 968. A convenient form of water frangible closure comprises a water soluble adhesive disposed along and sealing one edge of a pouch formed of a water impermeable polymeric film such as polyethylene or polypropylene.

40 [0119] In a variant of the bag or container product form, laminated sheet products can be employed in which a central flexible layer is impregnated and/or coated with a composition and then one or more outer layers are applied to produce a fabric-like aesthetic effect. The layers may be sealed together so as to remain attached during use or may separate on contact with water to facilitate the release of the coated or impregnated material.

45 [0120] An alternative laminate form comprises one layer embossed or deformed to provide a series of pouch-like containers into each of which the detergent components are deposited in measured amounts, with a second layer overlying the first layer and sealed thereto in those areas between the pouch-like containers where the two layers are in contact. The components may be deposited in particulate, paste or molten form and the laminate layers should prevent egress of the contents of the pouch-like containers prior to their addition to water. The layers may separate or may remain attached together on contact with water, the only requirement being that the structure should permit rapid release of the contents of the pouch-like containers into solution. The number of pouch-like containers per unit area of substrate is a matter of choice but will normally vary between 500 and 25,000 per square metre.

50 [0121] Suitable materials which can be used for the flexible laminate layers in this aspect of the invention include, among others, sponges, paper and woven and non-woven fabrics.

[0122] However the preferred means of carrying out the washing process according to the present invention includes the use of a reusable dispensing device having walls that are permeable to liquid but impermeable to the solid com-

position.

[0122] Devices of this kind are disclosed in European Patent Application Publication Nos. 0 343 069 and 0 344 070. The latter Application discloses a device comprising a flexible sheet in the form of a bag extending from a support ring defining an orifice, the orifice being adapted to admit to the bag sufficient product for one washing cycle in a washing cycle. A portion of the washing medium flows through the orifice into the bag, dissolves the product, and the solution then passes outwardly through the orifice into the washing medium. The support ring is provided with a masking arrangement to prevent egress of wetted, undissolved, product, this arrangement typically comprising radially extending walls extending from a central boss in a spoked wheel configuration, or a similar structure in which the walls have a helical form.

10

EXAMPLES

[0123] The following examples illustrate the invention and facilitate its understanding.

[0124] The abbreviations for the individual ingredients have the following meaning :

15

LAS: sodium salt of linear dodecyl benzene sulfonate

TAS: sodium salt of tallow alcohol sulfate

AS: sodium salt of alkyl (C14 - C15) sulfate

AO: C12 - C14 alkyl dimethylamine oxide

20

FA45E7: fatty alcohol (C14 - C15) ethoxylated with about 7 moles of ethylene oxide

CAT: C12 alkyl trimethyl ammonium chloride

Clay: smectite clay

Zeolite 4A: sodium salt of zeolite 4A with average particle size between 1 - 10 micrometer

SKS-6: crystalline layered silicate (Hoechst)

25

Copolymer AA/MA: copolymer of acrylic acid and maleic acid

PAA: polyacrylic acid MW 1000 -> 10000

CMC: carboxymethylcellulose

Phosphonate: sodium salt of ethylenediamine tetramethylene phosphonic acid

EDTA: sodium salt of ethylenediamine tetra acetate

30

PB1: NaBO₂.H₂O₂

PB4: NaBO₂.H₂O₂.3H₂O

TAED: tetra acetyl ethylene diamine

NOBS: - nonanoyl oxybenzene sodium sulfonate

P.A.: sulphonated zinc phthalocyanine

35

Silicate (R = n): SiO₂ / Na₂O = n

Amylase: Termamyl 60T (Novo-Nordisk)

Lipase: Lipolase 100T (Novo-Nordisk)

Protease: Savinase 4T (Novo-Nordisk)

SSS : Suds Suppressing System (silica/silicone mixture)

40

EXAMPLE I

Criticality of the cellulase performance parameter of claim 1

45

[0125] The following test was conducted :

Test conditions :

[0126]

50

Washing temperature : 60°C (heat up cycle)

Washing time : 40 min.

pH = 7.5

Water hardness : 4 mmol/L

55

Detergent concentration : 1%

Detergent composition : cfr. EPA 350 098 ex. 1

Cellulases :

EP 0 495 257 B1

- 1) Celluzyme® supplied by Novo Nordisk = reference
- 2) 43kD endoglucanase = cellulase according to the invention

Test Results :

5

[0127]

		% C14-CMC Removal by Cellulase
10	Detergent without cellulase (=reference)	0
	Detergent + Celluzyme®	
15	0.25 mg protein/L	below 3
	0.9 mg protein/L	10
	1.5 mg protein/L	12.7
	3.0 mg protein/L	17.7
	4.5 mg protein/L	21.5
	Detergent + 43kD endoglucanase	
20	0.3 mg protein/L	20.3
	0.25 mg protein/L	18.5

Discussion of the results :

25

[0128] The above data clearly demonstrate the criticality of the claimed parameter for the cellulases of the invention over the commercially available Celluzyme.

EXAMPLE II.

30

[0129] The following base compositions were prepared :

COMPOSITIONS: (all levels in % by weight)			
	Compact Detergent	Non-compact Detergent	
35	LAS	9.40	6.27
	TAS	3.00	2.00
	FA45E7	2.65	1.77
40	Na citrate/citric acid	18.50	12.33
	Zeolite 4A	32.65	21.77
	Copolymer AA/MA	4.90	3.27
	Phosphonate	0.19	0.13
	Na carbonate	3.00	2.00

45

COMPOSITIONS: (all levels in % by weight)			
	Compact Detergent	Non-compact Detergent	
50	Silicate (R = 2)	2.90	1.93
	Protease	1.62	1.08
	Sulfate	4.50	30.00
55	SSS	0.40	0.27
	Minors + water	balance to 100%	

(continued)

COMPOSITIONS: (all levels in % by weight)		
	Compact Detergent	Non-compact Detergent
Density (g/L at 20°C)	680	415
Recommended product usage (g/wash)	120	180

10 Color Rejuvenation Testing

Test conditions :

[0130]

15 Launderometer equipment
 Washing temperature : 40°C
 Washing time : 3h
 Number of wash cycles : 2
 20 pH = 8.2 non-compact detergent
 8.5 compact detergent
 Water hardness : 15gr./US gal.
 Detergent concentration :

25 0.75% for non-compact detergent
 0.66% for compact detergent

Test fabric : worn blue pyjama cotton
 (90/10 cotton/Polyester)

30 Cellulases :

1) Celluzyme® supplied by Novo Nordisk (= reference)
 2) 43kD endoglucanase = cellulase according to the present invention

35 Wash test : Swatches of 8g of worn blue pyjama fabric were treated with the different wash solutions. After tumble drying, the fabrics were graded for colour clarification effects by direct comparison of the two different detergent matrices at equal cellulase level. Visual grading by expert judges using a 0 to 4 scale was preferred. (0 stands for no difference and 4 stands for very big difference.)

40 Test Results :I) Non-Compact Detergent

[0131]

	PSU	mg protein/PSU
NO cellulase	0	
Celluzyme 138 mg protein/L	+ 2.3	60
43kD endoglucanase 18.6 mg protein/L	+ 2.2	8.5

II) Compact Detergent

[0132]

	PSU	mg protein/PSU
NO cellulase	0	

EP 0 495 257 B1

(continued)

	PSU	mg protein/PSU
5	Celluzyme 165 mg protein/L 43kD endoglucanase 3.4 mg protein/	+ 3.8 + 3.4
		43 1.0

LSD (Least Significant Difference) = 0.5 PSU

[0133] From the mg protein/PSU result, the following efficiency factors were calculated :

10 Efficiency factor of 43kD endoglucanase versus Celluzyme :

[0134]

	in Non Compact Detergent	in Compact Detergent
15	60/8.5 = 7	43/1.0 = 43

Efficiency factor in Compact Detergent versus in Non Compact Detergent

20 [0135]

	of Celluzyme	of 43kD endoglucanase
25	60/43 = 1.4	8.5/1 = 8.5

Conclusions :

30 [0136] The above results show a cellulase selected according to the present invention is 43 times more effective than a state-of-the-art cellulase in the claimed compact matrix. Furthermore, the above results show that the performance enhancement due to the claimed compact matrix seen with the selected cellulases is surprisingly much higher than what can be obtained with a state-of-the-art cellulase.

EXAMPLE III.

35 CLAY SOIL REMOVAL TESTING

[0137] Cellulase enzymes also are very efficient in removing clay stains from fabrics. This particular performance characteristic has been checked for a 43kD endoglucanase in the two detergent compositions given in example II.

40 Conditions:

[0138]

Linitest equipment

45 60C wash (heat up cycle)

Wash time: 40 min.

Water hardness: Brussels city water

Detergent concentrations:

50 0.66% for the Compact detergent

1.0% for the non compact detergent

Cellulase concentrations: 1.55, 3.10, 4,65 and 6.2mg enzyme protein / L wash liquor.

55 Wash test:

[0139] Muslin cotton fabric was soiled with naturally-derived clays of two different locations (US, UK). Cellulase performance was evaluated by comparing the clay stains washed at equal cellulase level in the two different detergent

EP 0 495 257 B1

compositions. The visual grading scale used in example II was again preferred.

Results:

5 [0140]

10	Cellulase level: (mg enz. prot. / L wash liquor)	1.55	3.1	4.7	6.2
Compact detergent					
15	US clay	+ 1.50	+ 2.50	+ 2.00	+ 1.50
	UK clay	+ 0.50	+ 1.00	+ 1.50	+ 2.50
	Non compact detergent (= reference)	0	0	0	0

20 LSD (least significant difference) = 0.42 at 95% confidence.

[0141] The clay stain removal performance of the cellulase selected according to the present invention, in the compact detergent composition of the invention is significantly superior to the performance of the same cellulase in the conventional, non compact detergent composition.

25 EXAMPLES IV-XI

[0142] The following compact detergent compositions are also prepared :

COMPACT DETERGENT COMPOSITIONS: (all levels in % by weight)									
EXAMPLE	IV	V	VI	VII	VIII	IX	X	XI	XII
LAS	9.40	12.50	11.00	---	7.58	7.58	8.20	6.50	-
TAS	3.00	---	---	---	2.43	2.43	2.65	3.25	3.90
AS	---	---	4.80	12.00	-	-	-	-	-
FA45E7	2.65	2.00	4.00	1.00	5.11	5.11	3.15	2.20	6.00
CAT	---	---	---	---	---	---	-	-	2.45
Coconut glucose amide	---	11.00	---	---	---	---	---	---	---
Tallow glucose amide	---	---	---	10.00	---	---	---	---	---
Na citrate/citric acid	20.50	29.50	18.00	18.00	---	5.00	23.50	12.00	15.00
Zeolite 4A	33.65	---	32.00	32.50	23.80	15.65	---	16.00	20.00
SKS-6	---	---	---	---	---	12.50	---	---	---
Copolymer AA/MA	4.90	---	4.10	5.00	5.60	2.90	3.50	3.45	3.45
PAA	---	5.70	---	---	---	---	1.50	---	---
Phosphonate	0.19	0.23	0.19	1.00	0.57	0.43	0.30	---	---
EDTA	---	---	---	---	0.25	-	-	0.32	0.32
Na carbonate / bicarbonate	2.00	12.00	3.28	2.50	17.30	8.00	2.50	9.90	9.90
Silicate (R = 2)	3.00	4.20	3.00	2.00	2.00	2.50	2.30	2.50	2.50
CMC	---	0.15	---	---	0.48	0.34	0.25	---	---
Clay	---	---	---	---	---	---	12.00	8.60	8.60
PB1	---	---	---	---	13.12	13.12	11.47	11.50	---
PB4	---	---	---	---	---	---	3.55	---	---
Percarbonate	---	---	---	---	---	---	---	---	12.00
TAED	---	---	---	---	5.70	5.70	2.47	3.20	-
NOBS	---	---	---	---	---	---	2.00	--	--

EP 0 495 257 B1

(continued)

COMPACT DETERGENT COMPOSITIONS: (all levels in % by weight)										
	EXAMPLE	IV	V	VI	VII	VIII	IX	X	XI	XII
5	P.A.	----	----	----	----	0.002	0.002	-	0.003	0.003
10	Protease	1.62	1.30	1.20	1.60	1.35	1.35	1.05	1.40	1.40
	Lipolase	----	----	0.40	0.30	-	0.20	-	0.30	0.30
15	Amylase	0.15	-	0.20	0.30	----	0.10	----	----	----
	Sulfate	2.54	3.79	2.38	2.45	1.50	1.50	2.23	3.45	3.45
	Brightener	---	0.27	0.27	0.27	0.24	0.24	0.24	0.24	0.24
	SSS	0.40	0.40	0.40	0.40	0.65	0.65	0.50	0.50	0.50
	Minors + water	balance to 100%								
	Cellutase	at levels so as to deliver 0.01 < X < 10 mg enzyme protein / wash liquor								

SEQUENCE DESCRIPTION: SEQ ID NO:1:

25

30

35

40

45

50

55

EP 0 495 257 B1

	GGATCCAAG ATG CGT TCC TCC CCC CTC CTC CCG TCC GCC GTG GCC	48
5	Met Arg Ser Ser Pro Leu Leu Pro Ser Ala Val Val Ala	
	-21 -20 -15 -10	
	GCC CTG CCG GTG TTG GCC CTT GCT GAT GGC AGG TCC ACC CGC TAC	96
	Ala Leu Pro Val Leu Ala Leu Ala Asp Gly Arg Ser Thr Arg Tyr	
	-5 1 5	
10	TGG GAC TGC TGC AAG CCT TCG TGC GGC TGG GCC AAG AAG GCT CCC GTG	144
	Trp Asp Cys Cys Lys Pro Ser Cys Gly Trp Ala Lys Lys Ala Pro Val	
	10 15 20	
15	AAC CAG CCT GTC TTT TCC TGC AAC GCC AAC TTC CAG CGT ATC ACG GAC	192
	Asn Gln Pro Val Phe Ser Cys Asn Ala Asn Phe Gln Arg Ile Thr Asp	
	25 30 35 40	
20	TTC GAC GCC AAG TCC GGC TGC GAG CCG GGC GGT GTC GCC TAC TCG TGC	240
	Phe Asp Ala Lys Ser Gly Cys Glu Pro Gly Gly Val Ala Tyr Ser Cys	
	45 50 55	
25	GCC GAC CAG ACC CCA TGG GCT GTG AAC GAC GAC TTC GCG CTC GGT TTT	288
	Ala Asp Gln Thr Pro Trp Ala Val Asn Asp Asp Phe Ala Leu Gly Phe	
	60 65 70	
	GCT GCC ACC TCT ATT GCC GGC AGC AAT GAG GCG GGC TGG TGC TGC GCC	336
	Ala Ala Thr Ser Ile Ala Gly Ser Asn Glu Ala Gly Trp Cys Cys Ala	
	75 80 85	
30	TGC TAC GAG CTC ACC TTC ACA TCC GGT CCT GTT GCT GGC AAG AAG ATG	384
	Cys Tyr Glu Leu Thr Phe Thr Ser Gly Pro Val Ala Gly Lys Lys Met	
	90 95 100	
35	GTC GTC CAG TCC ACC AGC ACT GGC GGT GAT CTT GGC AGC AAC CAC TTC	432
	Val Val Gln Ser Thr Ser Thr Gly Gly Asp Leu Gly Ser Asn His Phe	
	105 110 115 120	
40	GAT CTC AAC ATC CCC GGC GGC GTC GGC ATC TTC GAC GGA TGC ACT	480
	Asp Leu Asn Ile Pro Gly Gly Val Gly Ile Phe Asp Gly Cys Thr	
	125 130 135	

45

50

55

EP 0 495 257 B1

	CCC CAG TTC GGC GGT CTG CCC GGC CAG CGC TAC GGC GGC ATC TCG TCC Pro Gln Phe Gly Gly Leu Pro Gly Gln Arg Tyr Gly Gly Ile Ser Ser	528
5	140 145 150	
	CGC AAC GAG TGC GAT CGG TTC CCC GAC GCC CTC AAG CCC GGC TGC TAC Arg Asn Glu Cys Asp Arg Phe Pro Asp Ala Leu Lys Pro Gly Cys Tyr	576
	155 160 165	
10	TGG CGC TTC GAC TGG TTC AAG AAC GCC GAC AAT CCG AGC TTC AGC TTC Trp Arg Phe Asp Trp Phe Lys Asn Ala Asp Asn Pro Ser Phe Ser Phe	624
	170 175 180	
15	CGT CAG GTC CAG TGC CCA GCC GAG CTC GTC GCT CGC ACC GGA TGC CGC Arg Gln Val Gln Cys Pro Ala Glu Leu Val Ala Arg Thr Gly Cys Arg	672
	185 190 195 200	
20	CGC AAC GAC GAC GGC AAC TTC CCT GCC GTC CAG ATC CCC TCC AGC AGC Arg Asn Asp Asp Gly Asn Phe Pro Ala Val Gln Ile Pro Ser Ser Ser	720
	205 210 215	
	ACC AGC TCT CCG GTC AAC CAG CCT ACC AGC ACC AGC ACC ACG TCC ACC Thr Ser Ser Pro Val Asn Gln Pro Thr Ser Thr Ser Thr Ser Thr	768
	220 225 230	
25	TCC ACC ACC TCG AGC CCG CCA GTC CAG CCT ACG ACT CCC AGC GGC TGC Ser Thr Thr Ser Ser Pro Pro Val Gln Pro Thr Thr Pro Ser Gly Cys	816
	235 240 245	
30	ACT GCT GAG AGG TGG GCT CAG TGC GGC GGC AAT GGC TGG AGC GGC TGC Thr Ala Glu Arg Trp Ala Gln Cys Gly Gly Asn Gly Trp Ser Gly Cys	864
	250 255 260	
35	ACC ACC TGC GTC GCT GGC AGC ACT TGC ACG AAG ATT AAT GAC TGG TAC Thr Thr Cys Val Ala Gly Ser Thr Cys Thr Lys Ile Asn Asp Trp Tyr	912
	265 270 275 280	
	CAT CAG TGC CTG TAGACGCAGG GCAGCTTGAG GGCCCTTACTG GTGGCCGCAA His Gln Cys Leu	964
40	285	
	CGAAATGACA CTCCCCATCA CTGTATTAGT TCTTGTACAT AATTTCGTCA TCCCTCCAGG	1024
	GATTGTCACA TAAATGCAAT GAGGAACAAT GAGTAC	1060
45		

SEQUENCE DESCRIPTION: SEQ ID NO:2:

50

55

Met Arg Ser Ser Pro Leu Leu Pro Ser Ala Val Val Ala Ala Leu Pro
 -21 -20 -15 -10

5 Val Leu Ala Leu Ala Ala Asp Gly Arg Ser Thr Arg Tyr Trp Asp Cys
 -5 1 5 10

10 Cys Lys Pro Ser Cys Gly Trp Ala Lys Lys Ala Pro Val Asn Gln Pro
 15 20 25

15 Val Phe Ser Cys Asn Ala Asn Phe Gln Arg Ile Thr Asp Phe Asp Ala
 30 35 40

20 Lys Ser Gly Cys Glu Pro Gly Gly Val Ala Tyr Ser Cys Ala Asp Gln
 45 50 55

25 Thr Pro Trp Ala Val Asn Asp Asp Phe Ala Leu Gly Phe Ala Ala Thr
 60 65 70 75

30 Ser Ile Ala Gly Ser Asn Glu Ala Gly Trp Cys Cys Ala Cys Tyr Glu
 80 85 90

35 Leu Thr Phe Thr Ser Gly Pro Val Ala Gly Lys Lys Met Val Val Gln
 95 100 105

40 Ser Thr Ser Thr Gly Gly Asp Leu Gly Ser Asn His Phe Asp Leu Asn
 110 115 120

45 Ile Pro Gly Gly Val Gly Ile Phe Asp Gly Cys Thr Pro Gln Phe
 125 130 135

50 Gly Gly Leu Pro Gly Gln Arg Tyr Gly Gly Ile Ser Ser Arg Asn Glu
 140 145 150 155

55 Cys Asp Arg Phe Pro Asp Ala Leu Lys Pro Gly Cys Tyr Trp Arg Phe
 160 165 170

60 Asp Trp Phe Lys Asn Ala Asp Asn Pro Ser Phe Ser Phe Arg Gln Val
 175 180 185

65 Gln Cys Pro Ala Glu Leu Val Ala Arg Thr Gly Cys Arg Arg Asn Asp
 190 195 200

70 Asp Gly Asn Phe Pro Ala Val Gln Ile Pro Ser Ser Thr Ser Ser
 205 210 215

50

55

EP 0 495 257 B1

Pro Val Asn Gln Pro Thr Ser Thr Ser Thr Ser Thr Thr
 220 225 230 235

5 Ser Ser Pro Pro Val Gln Pro Thr Thr Pro Ser Gly Cys Thr Ala Glu
 240 245 250

10 Arg Trp Ala Gln Cys Gly Gly Asn Gly Trp Ser Gly Cys Thr Thr Cys
 255 260 265

Val Ala Gly Ser Thr Cys Thr Lys Ile Asn Asp Trp Tyr His Gln Cys
 270 275 280

15 Leu

SEQUENCE DESCRIPTION: SEQ ID NO:3:

20 GAATTCCGCGG CCGCTCATTCA ACTTCATTCA TTCTTTAGAA TTACATACAC TCTCTTTCAA 60

25 AACAGTCACT CTTAAACAA ACAACTTT GCAACA ATG CGA TCT TAC ACT CTT 114
 Met Arg Ser Tyr Thr Leu
 1 5

30 CTC GCC CTG GCC GGC CCT CTC GCC GTG AGT GCT GCT TCT GGA AGC GGT 162
 Leu Ala Leu Ala Gly Pro Leu Ala Val Ser Ala Ala Ser Gly Ser Gly
 10 15 20

35 CAC TCT ACT CGA TAC TGG GAT TGC TGC AAG CCT TCT TGC TCT TGG AGC 210
 His Ser Thr Arg Tyr Trp Asp Cys Cys Lys Pro Ser Cys Ser Trp Ser
 25 30 35

40 GGA AAG GCT GTC AAC GCC CCT GCT TTA ACT TGT GAT AAG AAC GAC 258
 Gly Lys Ala Ala Val Asn Ala Pro Ala Leu Thr Cys Asp Lys Asn Asp
 40 45 50

45 AAC CCC ATT TCC AAC ACC AAT GCT GTC AAC GGT TGT GAG GGT GGT GGT 306
 Asn Pro Ile Ser Asn Thr Asn Ala Val Asn Gly Cys Glu Gly Gly
 55 60 65 70

50 TCT GCT TAT GCT TGC ACC AAC TAC TCT CCC TGG GCT GTC AAC GAT GAG 354
 Ser Ala Tyr Ala Cys Thr Asn Tyr Ser Pro Trp Ala Val Asn Asp Glu
 75 80 85

55 CTT GCC TAC GGT TTC GCT ACC AAG ATC TCC GGT GGC TCC GAG GCC 402
 Leu Ala Tyr Gly Phe Ala Ala Thr Lys Ile Ser Gly Gly Ser Glu Ala
 90 95 100

EP 0 495 257 B1

	AGC TGG TGC TGT GCT TGC TAT GCT TTG ACC TTC ACC ACT GGC CCC GTC Ser Trp Cys Cys Ala Cys Tyr Ala Leu Thr Phe Thr Thr Gly Pro Val 105 110 115	450
5	AAG GGC AAG AAG ATG ATC GTC CAG TCC ACC AAC ACT GGA GGT GAT CTC Lys Gly Lys Lys Met Ile Val Gln Ser Thr Asn Thr Gly Gly Asp Leu 120 125 130	498
10	GGC GAC AAC CAC TTC GAT CTC ATG ATG CCC GGC GGT GGT GTC GGT ATC Gly Asp Asn His Phe Asp Leu Met Met Pro Gly Gly Val Gly Ile 135 140 145 150	546
15	TTC GAC GGC TGC ACC TCT GAG TTC GGC AAG GCT CTC GGC GGT GCC CAG Phe Asp Gly Cys Thr Ser Glu Phe Gly Lys Ala Leu Gly Gly Ala Gln 155 160 165	594
20	TAC GGC GGT ATC TCC TCC CGA AGC GAA TGT GAT AGC TAC CCC GAG CTT Tyr Gly Gly Ile Ser Ser Arg Ser Glu Cys Asp Ser Tyr Pro Glu Leu 170 175 180	642
	CTC AAG GAC GGT TGC CAC TGG CGA TTC GAC TGG TTC GAG AAC GCC GAC Leu Lys Asp Gly Cys His Trp Arg Phe Asp Trp Phe Glu Asn Ala Asp 185 190 195	690
25	AAC CCT GAC TTC ACC TTT GAG CAG GTT CAG TGC CCC AAG GCT CTC CTC Asn Pro Asp Phe Thr Phe Glu Gln Val Gln Cys Pro Lys Ala Leu Ile 200 205 210	738
30	GAC ATC AGT GGA TGC AAG CGT GAT GAC GAC TCC AGC TTC CCT GCC TTC Asp Ile Ser Gly Cys Lys Arg Asp Asp Asp Ser Ser Phe Pro Ala Phe 215 220 225 230	786
35	AAG GTT GAT ACC TCG GCC AGC AAG CCC CAG CCC TCC AGC TCC GCT AAG Lys Val Asp Thr Ser Ala Ser Lys Pro Gln Pro Ser Ser Ala Lys 235 240 245	834
40	AAG ACC ACC TCC GCT GCT GCC GCT CAG CCC CAG AAG ACC AAG GAT Lys Thr Thr Ser Ala Ala Ala Ala Gln Pro Gln Lys Thr Lys Asp 250 255 260	882
	TCC GCT CCT GTT GTC CAG AAG TCC TCC ACC AAG CCT GCC GCT CAG CCC Ser Ala Pro Val Val Gln Lys Ser Ser Thr Lys Pro Ala Ala Gln Pro 265 270 275	930
45	GAG CCT ACT AAG CCC GCC GAC AAG CCC CAG ACC GAC AAG CCT GTC GCC Glu Pro Thr Lys Pro Ala Asp Lys Pro Gln Thr Asp Lys Pro Val Ala 280 285 290	978
50	ACC AAG CCT GCT GCT ACC AAG CCC GTC CAA CCT GTC AAC AAG CCC AAG Thr Lys Pro Ala Ala Thr Lys Pro Val Gln Pro Val Asn Lys Pro Lys 295 300 305 310	1026
55	ACA ACC CAG AAG GTC CGT GGA ACC AAA ACC CGA GGA AGC TGC CCG GCC Thr Thr Gln Lys Val Arg Gly Thr Lys Thr Arg Gly Ser Cys Pro Ala 315 320 325	1074

EP 0 495 257 B1

AAG ACT GAC GCT ACC GCC AAG GCC TCC GTT GTC CCT GCT TAT TAC CAG Lys Thr Asp Ala Thr Ala Lys Ala Ser Val Val Pro Ala Tyr Tyr Gln 330 335 340	1122
5 TGT GGT GGT TCC AAG TCC GCT TAT CCC AAC GGC AAC CTC GCT TGC GCT Cys Gly Gly Ser Lys Ser Ala Tyr Pro Asn Gly Asn Leu Ala Cys Ala 345 350 355	1170
10 ACT GGA AGC AAG TGT GTC AAG CAG AAC GAG TAC TAC TCC CAG TGT GTC Thr Gly Ser Lys Cys Val Lys Gln Asn Glu Tyr Tyr Ser Gln Cys Val 360 365 370	1218
15 CCC AAC TAAATGGTAG ATCCATCGGT TGTGGAAGAG ACTATGCGTC TCAGAAGGGA Pro Asn 375	1274
20 TCCTCTCATG AGCAGGGTTG TCATTGTATA GCATGGCATC CTGGACCAAG TGTTGGACCC	1334
25 TTGTTGTACA TAGTATATCT TCATTGTATA TATTTAGACA CATAGATAGC CTCTTGTCAG CGACAACTGG CTACAAAAGA CTTGGCAGGC TTGTTCAATA TTGACACAGT TTCCCTCCATA AAAAAAA AAAA 25	1394 1454 1473

SEQUENCE DESCRIPTION: SEQ ID NO:4:

30

35

40

45

50

55

Met Arg Ser Tyr Thr Leu Leu Ala Leu Ala Gly Pro Leu Ala Val Ser
 1 5 10 15

5 Ala Ala Ser Gly Ser Gly His Ser Thr Arg Tyr Trp Asp Cys Cys Lys
 20 25 30

10 Pro Ser Cys Ser Trp Ser Gly Lys Ala Ala Val Asn Ala Pro Ala Leu
 35 40 45

15 Thr Cys Asp Lys Asn Asp Asn Pro Ile Ser Asn Thr Asn Ala Val Asn
 50 55 60

20 Gly Cys Glu Gly Gly Ser Ala Tyr Ala Cys Thr Asn Tyr Ser Pro
 65 70 75 80

25 Trp Ala Val Asn Asp Glu Leu Ala Tyr Gly Phe Ala Ala Thr Lys Ile
 85 90 95

30 Ser Gly Gly Ser Glu Ala Ser Trp Cys Cys Ala Cys Tyr Ala Leu Thr
 100 105 110

35 Phe Thr Thr Gly Pro Val Lys Gly Lys Lys Met Ile Val Gln Ser Thr
 115 120 125

40 Asn Thr Gly Gly Asp Leu Gly Asp Asn His Phe Asp Leu Met Met Pro
 130 135 140

45 Gly Gly Gly Val Gly Ile Phe Asp Gly Cys Thr Ser Glu Phe Gly Lys
 145 150 155 160

50 Ala Leu Gly Gly Ala Gln Tyr Gly Gly Ile Ser Ser Arg Ser Glu Cys
 165 170 175

55 Asp Ser Tyr Pro Glu Leu Leu Lys Asp Gly Cys His Trp Arg Phe Asp
 180 185 190

60 Trp Phe Glu Asn Ala Asp Asn Pro Asp Phe Thr Phe Glu Gln Val Gln
 195 200 205

65 Cys Pro Lys Ala Leu Leu Asp Ile Ser Gly Cys Lys Arg Asp Asp Asp
 210 215 220

70 Ser Ser Phe Pro Ala Phe Lys Val Asp Thr Ser Ala Ser Lys Pro Gln
 225 230 235 240

Pro Ser Ser Ser Ala Lys Lys Thr Thr Ser Ala Ala Ala Ala Ala Gln
 245 250 255

5 Pro Gln Lys Thr Lys Asp Ser Ala Pro Val Val Gln Lys Ser Ser Thr
 260 265 270

10 Lys Pro Ala Ala Gln Pro Glu Pro Thr Lys Pro Ala Asp Lys Pro Gln
 275 280 285

Thr Asp Lys Pro Val Ala Thr Lys Pro Ala Ala Thr Lys Pro Val Gln
 290 295 300

15 Pro Val Asn Lys Pro Lys Thr Thr Gln Lys Val Arg Gly Thr Lys Thr
 305 310 315 320

Arg Gly Ser Cys Pro Ala Lys Thr Asp Ala Thr Ala Lys Ala Ser Val
 325 330 335

20 Val Pro Ala Tyr Tyr Gln Cys Gly Gly Ser Lys Ser Ala Tyr Pro Asn
 340 345 350

25 Gly Asn Leu Ala Cys Ala Thr Gly Ser Lys Cys Val Lys Gln Asn Glu
 355 360 365

Tyr Tyr Ser Gln Cys Val Pro Asn
 370 375

30

Claims

35 1. A granular detergent composition comprising surface-active agent, builder and cellulase **characterized in that** said cellulase provides at least 10% removal of immobilized radioactive labelled carboxymethylcellulose according to the C14CMC-method, as described herein, at 25×10^{-6} % by weight of cellulase protein in the laundry test solution, and said granular detergent composition containing no more than 15% by weight of inorganic filler salt, and said granular detergent composition having a density of 550 to 950 g/litre of composition.

40 2. A granular detergent composition according to claim 1 **characterized in that** the cellulase compound consists essentially of a homogeneous endoglucanase component which is immunoreactive with a monoclonal antibody raised against a partially purified about $\approx 43\text{kD}$ endoglucanase derived from *Humicola insolens*, DSM 1800, or which is homologous to said $\approx 43\text{kD}$ endoglucanase.

45 3. A detergent composition according to claim 2 wherein the endoglucanase component of said cellulase has an isoelectric point of about 5.1.

50 4. A detergent composition in accordance with any of the previous claims wherein the level of the cellulase is such that the amount of enzyme protein to be delivered in the wash solution is from 0.005 to 40 mg/liter of wash solution, preferably 0.01 to 10 mg/liter of wash solution.

55 5. A detergent composition according to any of the previous claims wherein said inorganic filler salt is selected from alkali and alkaline-earth metal salts of sulphate and chloride.

6. A detergent composition in accordance with any of the previous claims which does not contain more than 10% by wt of inorganic filler salt.

7. A detergent composition in accordance with claim 6 which does not contain more than 5% by wt of inorganic filler salt.
8. A detergent composition according to any of the previous claims which has a density of 650 to 850 g/liter.
9. A detergent composition according to any of the previous claims which is free of phosphate compounds, and wherein said builder is selected from aluminosilicate ion exchangers, citrates, carbonates and mixtures thereof.
10. A granular detergent composition according to claim 1 characterized in that the cellulase compound is an endoglucanase enzyme having the amino acid sequence shown in the appended sequence listing ID#2, or is a homologue thereof exhibiting endoglucanase activity.
11. A detergent composition according to claim 10 wherein said endoglucanase enzyme is producible by a species of Humicola, e.g. Humicola insolens.
12. A granular detergent composition according to claim 1 characterized in that the cellulase compound is an endoglucanase enzyme having the amino acid sequence shown in the appended sequence listing ID#4, or is a homologue thereof exhibiting endoglucanase activity.
13. A detergent composition according to claim 10 wherein said endoglucanase enzyme is producible by a species of Fusarium, e.g. Fusarium oxysporum.
14. A detergent composition according to claim 10-13 wherein said enzyme is produced by a DNA construct comprising a DNA sequence encoding the enzyme, as shown in the appended sequence listings ID # 1 or ID#3
15. A detergent composition according to claims 10, 12 and 14, wherein said endoglucanase enzyme is producible within a strain of a fungus such as Trichoderma or Aspergillus, preferably Aspergillus oryzae or Aspergillus niger, or a yeast cell belonging to a strain of Hansenula or Saccharomyces, e.g. a strain of Saccharomyces cerevisiae.
16. A detergent composition according to claims 10, 12 and 14, wherein said endoglucanase enzyme is producible within a strain of a bacterium, e.g. Bacillus, Streptomyces or E. coli.
17. A process for washing fabrics in a washing machine wherein an amount of from 15 to 170 g of a detergent composition according to claims 1-16 is used for the main wash cycle.
18. A process for washing fabrics according to claim 17 wherein said amount of detergent composition is put in a container able to release the composition at the start of the wash cycle, and said container is placed in the drum of the washing machine, together with the fabrics to be washed.

40 Patentansprüche

1. Eine granulierte Reinigungsmittelzusammensetzung, umfassend oberflächenaktives Agens, Builder und Cellulase, dadurch gekennzeichnet, daß genannte Cellulase die Entfernung, gemäß der hierin beschriebenen C14CMC-Methode, von mindestens 10% immobilisierter, radioaktiv markierter Carboxymethylcellulose bei 25×10^{-6} Gew.-% an Cellulaseprotein in der Waschtestlösung erbringt, wobei die genannte granulierte Reinigungsmittelzusammensetzung nicht mehr als 15 Gew.-% an anorganischem Füllmittelsalz enthält, und die genannte granulierte Reinigungsmittelzusammensetzung eine Dichte von 550 bis 950 g pro Liter Zusammensetzung aufweist.
2. Eine granulierte Reinigungsmittelzusammensetzung nach Anspruch 1, dadurch gekennzeichnet, daß die Cellulaseverbindung im wesentlichen aus einer homogenen Endoglucanhydrolasekomponente besteht, welche immunreakтив mit einem monoklonalen Antikörper reagiert, der gegen eine teilweise gereinigte, ungefähr 43 kD Endoglucanhydrolase aus Humicola insolens DSM 1800 gebildet wurde oder welche homolog ist zur genannten, ungefähr 43kD Endoglucanhydrolase.
3. Eine Reinigungsmittelzusammensetzung nach Anspruch 2, bei der die Endoglucanhydrolasekomponente genann-

ter Cellulase einen isoelektrischen Punkt von ungefähr 5,1 hat.

4. Eine Reinigungsmittelzusammensetzung gemäß mindestens einem der vorgenannten Ansprüche, bei der der Gehalt an Cellulase so beschaffen ist, daß die Menge an Enzymprotein, die in die Waschlösung eingebracht werden soll, von 0,005 bis 40 mg/Liter Waschlösung reicht, vorzugsweise von 0,01 bis 10 mg/ Liter Waschlösung.
5
5. Eine Reinigungsmittelzusammensetzung gemäß mindestens einem der vorgenannten Ansprüche, bei der genanntes anorganisches Füllmittelsalz aus Alkaliund Erdalkalimetallsulfat- und Chloridsalzen ausgewählt ist.
10
6. Eine Reinigungsmittelzusammensetzung gemäß mindestens einem der vorgenannten Ansprüche, welche nicht mehr als 10 Gew.-% an anorganischem Füllmittelsalz enthält.
15
7. Eine Reinigungsmittelzusammensetzung nach Anspruch 6, welche nicht mehr als 5 Gew.-% an anorganischem Füllmittelsalz enthält.
15
8. Eine Reinigungsmittelzusammensetzung gemäß mindestens einem der vorgenannten Ansprüche, welche eine Dichte von 650 bis 850 g/Liter besitzt.
20
9. Eine Reinigungsmittelzusammensetzung gemäß mindestens einem der vorgenannten Ansprüche, welche frei ist an Phosphatverbindungen und worin genannter Builder ausgewählt ist aus Aluminosilicationaustauschern, Citraten, Karbonaten und Mischungen daraus.
25
10. Eine granulierte Reinigungsmittelzusammensetzung nach Anspruch 1, **dadurch gekennzeichnet, daß** die Cellulaseverbindung ein Endoglucanhydrolaseenzym ist, das eine Aminosäuresequenz, die der im Anhang gezeigten Sequenzliste ID#2 entspricht oder ein Homologes davon darstellt und Endoglucanhydrolaseaktivität aufweist.
25
11. Eine Reinigungsmittelzusammensetzung nach Anspruch 10, worin genanntes Endoglucanhydrolaseenzym durch eine Spezies von Humicola, z.B. Humicola insolens hergestellt werden kann.
30
12. Eine granulierte Reinigungsmittelzusammensetzung nach Anspruch 1, **dadurch gekennzeichnet, daß** die Cellulaseverbindung ein Endoglucanhydrolasenzym ist, welches eine Aminosäuresequenz aufweist, entsprechend der im Anhang gezeigten Sequenzliste ID#4, oder ein Homologes davon ist und Endoglucanhydrolaseaktivität aufweist.
35
13. Eine Reinigungsmittelzusammensetzung nach Anspruch 10, worin besagtes Endoglucanhydrolaseenzym durch eine Spezies von Fusarium, z.B. Fusarium oxysporum herstellbar ist.
35
14. Eine Reinigungsmittelzusammensetzung nach Anspruch 10-13, worin besagtes Enzym durch ein DNA-Konstrukt produziert wird, welches eine DNA-Sequenz, wie im Anhang unter Sequenzliste ID#1 oder ID#3 gezeigt, besitzt, die für das Enzym kodiert.
40
15. Eine Reinigungsmittelzusammensetzung nach den Ansprüchen 10, 12 und 14, bei der genanntes Endoglucanhydrolaseenzym durch einen Pilzstamm wie z.B. Trichoderma oder Aspergillus, vorzugsweise Aspergillus oryzae oder Aspergillus niger, oder eine Hefezelle, die zu einem Stamm von Hansenula oder Saccharomyces gehört, beispielsweise ein Stamm von Saccharomyces cerevisiae, produziert werden kann.
45
16. Eine Reinigungsmittelzusammensetzung nach den Ansprüchen 10, 12 und 14, bei der genanntes Endoglucanhydrolaseenzym durch einen Bakterienstamm produziert werden kann, beispielsweise Bacillus, Streptomyces oder E.coli.
50
17. Ein Verfahren zum Waschen von Textilien in einer Waschmaschine, bei dem eine Menge, reichend von 15 bis 170 g, einer Reinigungsmittelzusammensetzung nach Ansprüchen 1 bis 16 für den Hauptwaschgang benutzt wird.
55
18. Ein Verfahren zum Waschen von Textilien gemäß Anspruch 17, bei dem genannte Menge an Reinigungsmittelzusammensetzung in einen Behälter eingebracht wird, welcher die Zusammensetzung zu Beginn des Waschganges freisetzen kann, und genannter Behälter in die Trommel der Waschmaschine zusammen mit den zu waschenden Textilien gelegt wird.

Revendications

1. Composition détergente granulaire comprenant un agent tensioactif, un adjuvant et une cellulase, **caractérisée en ce que** ladite cellulase permet d'éliminer au moins 10% de carboxyméthylcellulose radioactive marquée immobilisée, d'après la méthode C14CMC telle que décrite ici, à 25×10^{-6} % en poids de protéine de cellulase dans la solution d'essai de blanchisserie,
ladite composition détergente granulaire ne contenant pas plus de 15 % en poids de sel de charge minérale, et
ladite composition détergente granulaire ayant une concentration de 550 à 950 g/litre de composition.
2. Composition détergente granulaire selon la revendication 1, **caractérisée en ce que** le composé cellulase est essentiellement constitué par un constituant endoglucanase homogène qui est immunoréactif avec un anticorps monoclonal dirigé contre une endoglucanase d'environ ~43 kD partiellement purifiée obtenue à partir de *Humicola insolens*, DSM 1800, ou qui est homologue de ladite endoglucanase de ~43 kD.
3. Composition détergente selon la revendication 2, dans laquelle le constituant endoglucanase de ladite cellulase a un point isoélectrique d'environ 5,1.
4. Composition détergente selon l'une quelconque des revendications précédentes, dans laquelle la teneur en cellulase est telle que la quantité de protéine d'enzyme à délivrer dans la solution de lavage est de 0,005 à 40 mg/litre de solution de lavage, de préférence 0,01 à 10 mg/litre de solution de lavage.
5. Composition détergente selon l'une quelconque des revendications précédentes, dans laquelle ledit sel de charge minérale est choisi parmi les sels sulfates et chlorures de métaux alcalins et alcalino-terreux.
6. Composition détergente selon l'une quelconque des revendications précédentes, qui ne contient pas plus de 10% en poids de sel de charge minérale.
7. Composition détergente selon la revendication 6, qui ne contient pas plus de 5 % en poids de sel de charge minérale.
8. Composition détergente selon l'une quelconque des revendications précédentes, qui a une concentration de 650 à 850 g/litre.
9. Composition détergente selon l'une quelconque des revendications précédentes, qui est exempte de composés phosphates, et dans laquelle ledit adjuvant est choisi parmi les échangeurs d'ions aluminosilicates, les citrates, les carbonates et des mélanges de ceux-ci.
10. Composition détergente granulaire selon la revendication 1, **caractérisée en ce que** le composé cellulase est une enzyme endoglucanase ayant la séquence d'acides aminés présentée dans la liste de séquences annexée ID#2, ou est un homologue de celle-ci présentant l'activité d'une endoglucanase.
11. Composition détergente selon la revendication 10, dans laquelle ladite enzyme endoglucanase peut être produite par une espèce de *Humicola*, par exemple *Humicola insolens*.
12. Composition détergente granulaire selon la revendication 1, **caractérisée en ce que** le composé cellulase est une enzyme endoglucanase ayant la séquence d'acides aminés présentée dans la liste de séquences annexée ID#4, ou est un homologue de celle-ci présentant l'activité d'une endoglucanase.
13. Composition détergente selon la revendication 10, dans laquelle ladite enzyme endoglucanase peut être produite par une espèce de *Fusarium*, par exemple *Fusarium oxysporum*.
14. Composition détergente selon les revendications 10-13, dans laquelle ladite enzyme est produite par un produit d'assemblage d'ADN comprenant une séquence d'ADN codant pour l'enzyme, telle que présentée dans les listes de séquences annexées ID#1 ou ID#3
15. Composition détergente selon les revendications 10, 12 et 14, dans laquelle ladite enzyme endoglucanase peut être produite dans une souche d'un champignon tel que *Trichoderma* ou *Aspergillus*, de préférence *Aspergillus*

EP 0 495 257 B1

ozyzae ou *Aspergillus niger*, ou une cellule de levure appartenant à une souche de *Hansenula* ou *Saccharomyces*, par exemple une souche de *Saccharomyces cerevisiae*.

5 16. Composition détergente selon les revendications 10, 12 et 14, dans laquelle ladite enzyme endoglucanase peut être produite dans une souche d'une bactérie, par exemple *Bacillus*, *Streptomyces* ou *E. coli*.

17. Procédé pour laver des tissus dans une machine à laver, dans lequel une quantité de 15 à 170 g d'une composition détergente selon les revendications 1-16 est utilisée pour le cycle de lavage principal.

10 18. Procédé pour laver des étoffes selon la revendication 17, dans lequel ladite quantité d'une composition détergente est placée dans un récipient apte à libérer la composition au début du cycle de lavage, et ledit récipient est placé dans le tambour de la machine à laver, avec les étoffes à laver.

15

20

25

30

35

40

45

50

55